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Anti-*Saccharomyces cerevisiae* antibodies (ASCA) and autoimmune liver diseases

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SUMMARY

Antibodies to the baker's yeast *Saccharomyces cerevisiae* (ASCA), recently proposed as a serological marker of Crohn's disease, have also been detected in other autoimmune disorders. The aim of this study was to determine prevalence and clinical significance of ASCA in autoimmune liver disease. The presence of IgG and IgA ASCA was evaluated using a commercially available immunoassay in 215 patients with autoimmune liver disease (primary biliary cirrhosis, PBC, 123 cases; autoimmune hepatitis, AIH, 67 cases; primary sclerosing cholangitis, PSC, 25 cases), 48 with inflammatory bowel disease and 19 healthy blood donors. Anti neutrophil cytoplasmic antibodies with the perinuclear pattern (p-ANCA) were assessed by indirect immunofluorescence in PSC patients. The main clinical and biochemical parameters between ASCA-positive and negative patients were analysed and compared. ASCA are predominant in Crohn's disease (70%); among liver patients, PSC and AMA-negative PBC show the highest ASCA prevalence (53% and 44%). In PBC ASCA correlate with higher levels of circulating IgA ($P < 0{\cdot}05$). In PSC the detection of either ASCA or p-ANCA is neither associated with any clinical or biochemical feature, nor with an underlying inflammatory bowel disease. ASCA can not be considered an additional serological marker of autoimmune liver disease, but the possibility of detecting such a reactivity in autoimmune liver disorders should be considered; their correlation with elevated IgA in PBC suggests that ASCA may be an indirect sign of enhanced mucosal immunity; in PSC patients neither ASCA nor p-ANCA predict the occurrence of a concomitant inflammatory bowel disease.

Keywords autoimmune liver disease autoimmune hepatitis primary biliary cirrhosis autoantibodies

INTRODUCTION

In recent years the detection of humoral reactivity to the baker's yeast *Saccharomyces cerevisiae* (anti-*Saccharomyces cerevisiae* antibodies, ASCA) has been reported in inflammatory bowel diseases, particularly in patients with more aggressive clinical expression of Crohn's disease [1–3]. Although ASCA are not classical autoantibodies, their appearance seems to be favoured by a genetic predisposition [4].

Many studies have been carried out in inflammatory bowel disease patients in order to differentiate between ulcerative colitis and Crohn's on the basis of ASCA or antineutrophil cytoplasmic antibodies with the perinuclear pattern (p-ANCA) [5–7].

Recently, Reddy *et al.* [8] detected ASCA also in autoimmune liver disease, and reported a similar prevalence of such a reactivity (ranging from 9 to 22%) in patients with autoimmune and viral liver disorders, as well as in blood donors.

At the light of the epidemiological associations between inflammatory bowel disease (particularly ulcerative colitis) and primary sclerosing cholangitis (PSC) [9], a cholestatic liver disease with a likely autoimmune pathogenesis, we evaluated the clinical and immunological impact of ASCA on a large series of patients with different autoimmune liver disorders. Therefore, we analysed and correlated ASCA status with biochemical and immunological parameters in autoimmune liver patients, and evaluated the possibility that ASCA and p-ANCA may provide information on the presence and type of a potential underlying inflammatory bowel disease in PSC patients.

PATIENTS AND METHODS

Patients

Two hundred eighty-two patients were studied. Of these, 123 had PBC (17 were AMA-negative) diagnosed according to the criteria proposed by Taal *et al.* [10]. All AMA positive PBC patients

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reacted by western immunoblot with a 74-kD polypeptide, corresponding to the E2 component of the pyruvate dehydrogenase complex, and with additional proteins of 55 kD, 52 kD, 48 kD and 41 kD in different combinations [11]. None of the 17 AMA negative PBC sera reacted by western immunoblot with any polypeptide of the mitochondrial preparation. Sixty-seven patients had autoimmune hepatitis (AIH) diagnosed according to internationally agreed and recently revised criteria [12]. Of them, 30 were positive for antinuclear (ANA) and/or antismooth muscle antibodies (SMA) and were diagnosed as type 1 AIH. The remaining 37 patients were positive for liver/kidney microsomal antibody type 1 (LKM1) and/or liver cytosol antibody type 1 (LC1) and were diagnosed as type 2 AIH. Twenty-five patients had PSC, a diagnosis supported by the typical 'beading' appearance of the intra and/or extra-hepatic biliary tree on ERCP examination. In addition, sera from 48 patients with inflammatory bowel disease (23 with Crohn's and 25 with ulcerative colitis, respectively) and from 19 healthy blood donors were also tested as positive and negative controls.

Patients were grouped according to the ASCA status, and biochemical and immunological parameters such as alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma-glutamiltranspeptidase, total and direct bilirubin, albumin, gammaglobulins, prothrombin time, cholesterol, IgG, IgA and IgM were compared. All patients gave their informed consent to partecipate to the study

Methods

ANA, SMA, LKM1, LC1 and AMA were detected by indirect immunofluorescence, as previously described [13]. Briefly, sera diluted 1 : 40 in phosphate buffered saline (PBS) were tested on snap-frozen sections of rat liver, kidney and stomach. A fluorescein-conjugated secondary antibody directed against human immunoglobulins (Anti-Human Polyvalent Immunoglobulins IgA, IgG, IgM FITC Conjugate, Sigma ImmunoChemicals, St. Louis, MO, USA) was used diluted 1 : 100 in PBS. The patterns of reactivity were assessed under a fluorescence microscopy (Orthoplan, Leitz, Wetzlar, Germany). The immuno-morphological pattern of ANA-positive sera was evaluated further on commercially available HEp-2 cell lines (Kallestad, Chaska, MN, USA). The detection of LKM1 and LC1 was validated by immunoblot using human liver microsomal and cytosolic preparations, respectively: LKM1-positive sera reacted with a 50-kD microsomal protein corresponding to CYP2D6 [14], whereas LC1-positive sera recognized a 58-kD cytosolic protein [15] corresponding to formiminotransferase cyclodeaminase. AMA was characterized by western immunoblotting using as a source of antigens a mitochondrial preparation from bovine heart [11]. Detection of ANCA was performed by indirect immunofluorescence at 20× and 40× magnification on alcohol-fixed human neutrophils, with sample sera diluted 1 : 20, as previously described in detail [16].

The presence of ASCA (IgG and IgA class) was assessed by a commercially available ELISA kit (Inova Diagnostics, San Diego, CA). According to the manufacturer's instructions, an ASCA reactivity higher than 29 U/l (i.e. 20% above the cut-off) was considered strongly positive.

Statistical analysis

Comparison of categorical variables was performed using chi-square and Fisher test. The non parametric *T*-test was used for comparison of continuous data. Nominal variables were correlated by contingency table. A probability $P < 0{\cdot}05$ was considered significant.

RESULTS

Fifty-two (25%) of 215 autoimmune liver patients had ASCA of either IgA and/or IgG class. Of these, 18 (8%) were ASCA IgA and IgG positive, 13 (6%) were ASCA IgG positive/IgA negative, and 21 (10%) were ASCA IgG negative/IgA positive. Twelve out of 23 (52%) Crohn's patients were ASCA IgG and IgA positive, 16 were ASCA IgG positive/IgA negative and 16 out of 23 (52%) were IgG negative/IgA positive. The prevalence of ASCA in healthy blood donors was 5% (1 out of 19) for IgA, and 0% for IgG. All positive sera but 2 showed strong ASCA reactivity, as illustrated in Fig. 1.

ASCA prevalence for each disease is summarized in Table 1. IgG but not IgA circulating ASCA levels in Crohn's patients were higher than in autoimmune liver patients ($P < 0{\cdot}01$).

Among the patients with liver disease the following statistically significant differences were observed: type 1 AIH, PSC and AMA-negative PBC had IgG ASCA prevalence higher than AMA-positive PBC and blood donors ($P < 0{\cdot}01$); AMA-negative PBC had IgA ASCA prevalence higher than blood donors ($P = 0{\cdot}03$).

The presence of ASCA was not associated with any of the clinical or biochemical parameters analysed. In PBC, but not in PSC and AIH, ASCA-positive patients had higher IgA levels ($P < 0.05$), irrespective of their AMA status.

None of the ASCA-positive patients with PSC had Crohn's disease, while 3 had a diagnosis of ulcerative colitis; furthermore, the two PSC patients with Crohn's disease were ASCA-negative.

The correlation between ASCA and p-ANCA reactivity in PSC patients is reported in Table 2.

DISCUSSION

Within the heterogeneous spectrum of autoimmune liver disease, we are used to differentiating between 'hepatitic' (e.g. AIH) and 'cholestatic' forms (e.g. PBC, PSC). In AIH the autoimmune attack is directed exclusively against the hepatocyte, whereas the cholangiocyte is the target in PBC and PSC. However, new clinical and nosological entities, provisionally named 'variant' or 'overlap syndromes' [17,18], are being increasingly recognized, in which both the hepatocyte and the cholangiocyte are considered the target of the immuno-mediated reaction. Even if the autoantibody profile of AIH, PSC and PBC is well characterized and typically mutually exclusive, some autoreactivities are shared by different diseases, as in the case of the perinuclear antineutrophil cytoplasmic antibodies (p-ANCA), detectable both in PSC and in type 1 AIH [16].

In recent years ASCA have been extensively studied in inflammatory bowel disorders, and their presence has been significantly correlated with active Crohn's disease and, less often, with ulcerative colitis. Even if the aetiology of such diseases is largely unknown, autoimmune mechanisms are hypothesized to play a role.

In this study the high prevalence of ASCA in Crohn's patients is confirmed, particularly when IgA and IgG ASCA are considered together (Table 1).

Our results suggest that in all the autoimmune liver diseases analysed ASCA are often present, particularly in PSC and in AMA-negative PBC (44% and 53%, respectively).

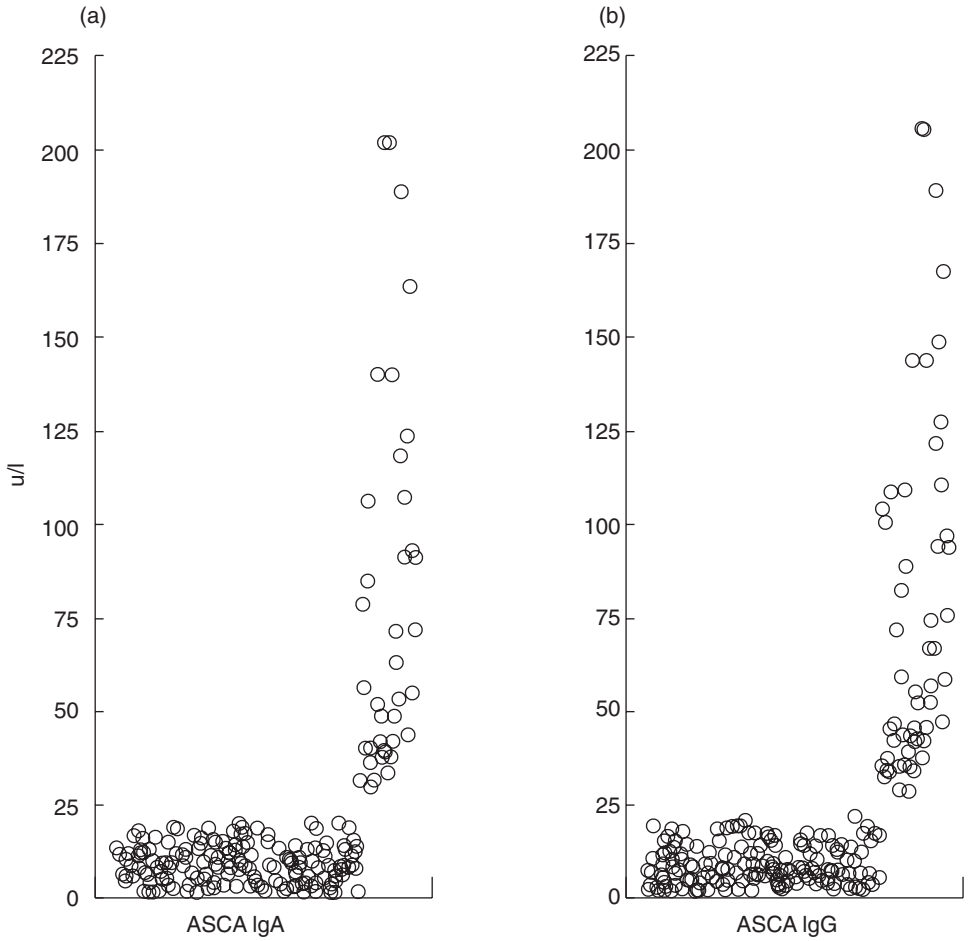

Fig. 1 Scattergram illustrating the distribution of ASCA reactivity to (a) IgA and (b) IgG in 215 patients with autoimmune liver disease, 48 with inflammatory bowel disease and 19 healthy blood donors.

Table 1. Distribution of ASCA reactivity in patients with autoimmune liver disease and inflammatory bowel disorders

	No. of patients	ASCA	ASCA IgA	ASCA IgG	ASCA IgA + IgG
AMA-pos. PBC	106	19 (18)	17 (16)	7 (7)	4 (4)
AMA-neg. PBC	17	9 (53)	6 (35)	6 (35)	3 (18)
Type 1 AIH	30	8 (27)	6 (20)	7 (23)	5 (17)
Type 2 AIH	37	4 (11)	2 (5)	4 (11)	2 (5)
PSC	25	11 (44)	8 (32)	7 (28)	4 (16)
Crohn's disease	23	16 (70)	12 (52)	16 (70)	12 (52)
Ulcerative colitis	25	9 (36)	5 (20)	7 (28)	5 (20)
Blood donors	19	1 (5)	1 (5)	0	0

AMA, antimitochondrial antibody; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis; PSC, primary sclerosing cholangitis.

Table 2. Relationship between ASCA reactivity and inflammatory bowel diseases associated with PSC

	ASCA IgA	ASCA IgG	p-ANCA
PSC (all cases)	8/25	7/25	10/25
PSC + CD (2 cases)	0/2	0/2	2/2
PSC + UC (5 cases)	2/5	4/5	3/5

PSC, primary sclerosing cholangitis; CD, Crohn disease; UC, ulcerative colitis.

Notwithstanding the elevated gammaglobulin levels usually detected in patients with autoimmune liver disease, it is highly unlikely that the ASCA reactivity might be attributed just to high immunoglobulin levels, since ASCA-positive and ASCA-negative patients had similar IgG and IgA values in the present series.

ASCA reactivity does not correlate with any of the biochemical parameter examined, therefore its presence does not seem to have a clinical impact on the autoimmune liver disease. The only immunological feature associated with ASCA are the elevated IgA levels observed in PBC patients. This finding could be a further manifestation of the enhanced mucosal-derived IgA secretion, a feature typical of PBC patients, in whom mucosal immunity appear to be over expressed, hence the detection of AMA of the IgA class in secretions such as saliva, urine [19–21].

In PSC patients, ASCA positivity does not predict the presence of a concomitant inflammatory bowel disease; however,

since the number of our PSC patients with associated bowel disorders is relatively low, it would be unwise to draw definite conclusions on such point.

At the light of their low sensitivity and specificity, ASCA can not be recommended as additional diagnostic tools in autoimmune liver disease, but the possibility to find them in association with diseases other than inflammatory bowel disease must be considered.

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